SUPPORT FOR THE AMENDMENTS

Applicants have added new Claims 62-74. Support for new Claims 62-72 can be found in Claims 51-61, as previously presented, and in Claim 3, as originally filed. Support for new Claims 73 and 74 can be found on page 22, last line, to page 23, line 2, of the specifcation.

No new matter has been added. Claims 51-74 are pending in this application.

REMARKS

The present claims relate to a method of transfection of DNA into cells, comprising contacting DNA with a fullerene derivative having 1 to 4 nitrogen-containing hydrophilic side chains or a salt thereof in the presence of cells.

The inventors have discovered that contacting DNA with a fullerene derivative having 1 to 4 nitrogen-containing hydrophilic side chains or a salt thereof in the presence of cells is particularly effective for carrying out the transfection of DNA into cells.

The cited references, even in combination, do not contain any disclosure which would suggest the presently claimed method. Specifically, none of these references contains any disclosure of ***contacting DNA*** with a fullerene derivative. Accordingly, these references cannot affect the patentability of the present claims.

The rejection of Claims 51-58 under 35 U.S.C. § 103(a) in view of U.S. Patent No. 5,310,669 (Richmond et al) in view of U.S. Patent No. 6,204,391 (Friedman et al) and WO 96-36631 (Murphy et al) is respectively traversed. Richmond et al discloses certain fullerene-

coated surfaces. This reference also discloses certain methods for introducing a substance such as DNA into a cell. However, the method disclosed in Richmond et al does not involve contacting the DNA with any fullerene derivative.

Instead, Richmond et al discloses irradiating the fullerene-caoted surface in the presence of oxygen to form reactive singlet oxygen which damages the cell membrane thereby increasing permeability:

> Methods for damaging the plasma membrane, for increasing cell membrane premability, and for introducing substances into a cell are also provided. Such methods comprise first attaching cells to a cell culture substrate having a fullerene-coated surface and maintaining the cells under conditions appropriate for cell growth. The cells are then illuminated with light in the presence of oxygen to damage the cell membrane and thereby increase cell membrane permeability. Subsequently, or prior to illumination, the cells are contacted with a substance to be introduced into the cells. The increased cell membrane permeability allows the substance to be introduced into the cell. The method is useful for transfecting a cell with, for example, a vector comprising DNA or RNA.

Richmond et al, col. 2, lines 11-26.

> Illumination of a fullerene in solution with absorbing light in the presence of molecular oxygen generates highly reactive singlet oxygen ($^1O_2$) by a semi-catalytic process without concurrent damage to the fullerene (Arbogast, J. W., et al., *J. Phys. Chem.* 95:11-12 (1991)). Substances, such as cells or macromolecules can therefore be attached to a fullerene-coated surface and illuminated with light in the presence of oxygen to induce $^1O_2$ damage. When cells are attached to a fullerene-coated surface, the reactivity of $^1O_2$ is such that it is unlikely to diffuse beyond the attached cell membrane (Moan, J., *J. Photochem. Photobiol. B: Biology* 6:343-344 (1990); Suwa, K., et al., *Biochem Biophys. Res. Comm.* 75:785-792 (1977)). Thus, the predominate oxidative

> reactions are likely to be peroxidations and cycloadditions of $^1O_2$ at carbon-to-carbon double bonds, resulting in increased cell membrane permeability. The length of illumination, intensity and wavelength of the light can be selected to quantitatively control the membrane induced damage. This technique is useful to study cell membrane composition e.g., cholesterol content, low- vs high-density lipoprotein interactions and the effect of oxidative damage on the cell membrane.
>
> In addition, selective increases in cell membrane permeability allow the introduction of substances (e.g., a vector) into the cell. Cells attached to a fullerene-coated surface can be cultured with a substance to be introduced into the cell prior to, simultaneously with, or following illumination with light in the presence of oxygen. For example, membrane porosity can be manipulated to accommodate the entry of vectors, transfecting DNA, antibodies and other proteins, DNA pool intermediates, a drug, etc.

Richmond et al, col. 3, line 65, to col. 4, line 29.

Thus, Richmond et al does not disclose or even remotely suggest "contacting DNA" with any fullerene compound at all, let alone a fullerene derivative having 1 to 4 nitrogen-containing hydrophilic side chains, as recited in the present claims. Likewise, this reference fails to suggest that fullerene derivatives having 1 to 4 nitrogen-containing hydrophilic side chains would be useful in any methods of transfection of DNA into cells.

Applicants submit that the secondary references cannot cure the deficiencies of Richmond et al. Specifically, Friedman et al is completely unconcerned with water soulble fullerene with certain antiviral activity. There is no disclosure or suggestion in Friedman et al of contacting DNA with any fullerene compounds. Instead, this reference is concerned with the binding of certain fullerene compounds with HIV-1 protease.

Likewise, Murphy et al is completely unconcerned with "contacting DNA" with any fullerene compounds. Rather, this reference is directed toward the preparation of multiply-substituted fullerenes. There is no disclosure of contacting any fullerene compounds with DNA or of any transfection of DNA into cells.

Moreover, there is no teaching in any of the cited references which would suggest using any of the fullerene derivatives of either Friedman et al or Murphy et al to prepare the surface of Richmond et al. Specifically, Richmond et al is concerned with preparing a fullerene-coated surface which possesses the ability to generate reactive singlet oxygen upon irradiation in the presence of oxygen. There is nothing in either Friedman et al or Murphy et al which would suggest that the fullerene derivatives of these secondary references would be useful for preparing such a surface.

Accordingly, the rejection of Claims 51-58 and 73 is improper and should be withdrawn.

In addition, the Examiner's attention is directed to newly added Claims 62-72 and 74, which require that the transfection be carried out by "compacting said DNA by contacting said DNA with said fullerene derivative or a salt thereof in the presence of cells." Certainly, there is no disclosure of compacting DNA by contacting the DNA with a fullerene derivative in any of the cited references. For these reasons, Claims 62-72 and 74 are surely patentable over the cited references.

Applicants submit that the application is now in condition for allowance, and early notification of such action is earnestly solicited.

Respectfully submitted,

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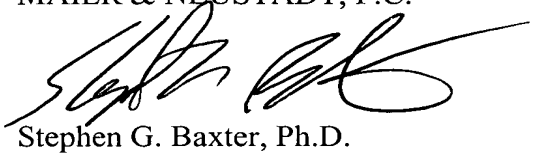

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